LOSSES OF CAROTENE (PRO-VITAMIN A) IN CURING ALFALFA HAY

by

FRIEDRICH EDWARD MEENEN

B. S., Kansas State College
of Agriculture and Applied Science, 1941

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1945

TABLE OF CONTENTS

INTRODUCTION

Alfalfa ranks high in importance among cultivated crops. Its value as a hay crop was emphasized by Morrison (1936) in his statement, "Alfalfa (Medicago sativa) stands at the head of the list of all common hay crops and is commonly taken as the ideal with which other hay is compared." In 1943, 722,000 acres of alfalfa were grown in Kansas.[1] A relatively small percentage of this acreage is being used by dehydrating and other alfalfa processing plants, and a very large percentage is being put up as hay and fed to livestock.

It appears that a study of the losses of carotene (pro-vitamin A) by common hay-making practices would be of considerable economic importance and might lead to improved practices by which hay of higher quality could be produced. It was thought that practices which would tend to conserve carotene might also tend to conserve the other important feeding constituents found in alfalfa hay.

The ultimate goal of this study was to determine the practice or practices which would be practical for farmers to use in hay-making to reduce carotene losses to a minimum.

LITERATURE REVIEW

The quality of alfalfa hay is dependent not only on its content of proteins, sugars, fats, and minerals, but also on its content of vitamins. Studies have been made on various treatments to find the practices which will yield the most carotene per unit of hay.

[1]Supplied in letter by Mr. H. L. Collins, State Agricultural Statistician for Kansas.

In 1940 Snyder and Moore made a study of alfalfa, bromegrass, corn leaves, oats, soybeans, sudan and sweet clover. They reported that the carotene content of these herbages is much greater during the earlier stages of growth than after they reach the stage of maturity at which they are usually harvested. Hauge (1934) reported that the vitamin A value of young alfalfa (10 to 12 inches high) was much greater than that of alfalfa in the bloom stage. Douglass, Tobiska and Vail (1933) found that alfalfa cut at the early bloom stage contains more carotene as a rule than that cut at other stages of growth, the method of curing being the same.

Kiesselbach and Anderson (1931) found that three-fourths of the protein of alfalfa was found in the leaves. Thus any curing process should be directed toward leaf conservation. No difference was found in time of curing or in quality of hay whether it was cut in the forenoon, noon, or afternoon, provided swath curing proceeded raking. They found further that raking immediately following the mower lengthened the curing period and lowered the color regardless of the type of rake or the size of windrow. Ordinarily under such conditions as prevail in Nebraska, cocking alfalfa hay as a part of the curing procedure was inadvisable.

That carotene is destroyed during the curing process was brought out by Russell, Taylor, and Chichester (1934). They reported that 80 percent of the original carotene content was lost during the first 24 hours after the hay was cut, the highest rate of destruction being during the daylight hours. Dexter and Moore (1937) tested curing alfalfa in the swath, windrow and cock. They reported that up until the time that hay was rather thoroughly wilted in the swath, carotene destruction was quite slow and

was lost at the same rate from the swath and windrow. They also found that windrow- and cock-cured hay were about equal in their carotene content when ready to barn, although the windrow-cured hay was ready two days before that cured in the cock, and that both windrow- and cock-cured hay contained about one and one-half times as much carotene as did that in the swath.

Woods, Atkeson, Willhousen and Johnson (1936) found that swath curing for three days, then cock cured and sweated in the stack for two and one-half to three months contained 116±9 rat units, while the same hay swath cured for one day, then cock cured and sweated in the stack contained 144±10 rat units. Cock-cured hay was compared with cock-cured and then sweated in the stack for 49 days. The former contained 233±20 rat units of vitamin A activity per gram, while the sweated hay contained 144±10 units.

Wallis (1942) found that during the first day there was not so much difference in the rate of destruction of carotene in the swath and small windrow, but after that destruction in the swath was more rapid. At the time hay is ready to haul, the windrow alfalfa may have up to twice as much carotene as that cured in the swath. He found also that it takes two to four days of good sunshine exposure to develop the maximum amount of vitamin D in alfalfa hay.

As an operation whereby the curing process may be stepped up, the hay crusher has been used to some extent. Vail, Tobiska, and Douglass (1936) found the vitamin A contents of crushed samples to be 45 and 87 percent higher than of the respective checks. Crushed hay dried in the sunlight compared favorably with oven-dried samples. Bechdel, Clyde, Cromer, and

Williams (1940) reported hay crushers as being a promising development for speeding up field curing of hay; by shortening the curing time it reduced weather hazard.

There is considerable destruction of carotene in alfalfa in storage, particularly during the warm months of the year. Smith (1936) found that, under conditions in Mesa, Arizona, baled alfalfa stored from August to November in a hay barn contained 50 percent less vitamin A than freshly baled alfalfa. No further destruction occurred until after January when rising temperatures stimulated destruction again.

Wallis (1942) reported that hays which contained 88 and 158 micrograms of carotene per gram of dry matter, after five months storage in the now still contained 16 and 28 micrograms per gram, respectively. Kane, Wiseman and Cary (1937) found that baled hays stored in a rather dark unheated barn when the outside temperature was 7.2° C. or less, lost carotene at a rate of about three percent per month; when the temperature was 7.2° to 18.9° C. the loss was 6.5 percent per month, and when the temperature was above 18.9° C. the loss of carotene increased rapidly.

Dehydration is becoming an important method of handling alfalfa. This process makes it available for use in many commercial feeds for livestock and poultry. Russell, Taylor, and Chichester (1934) found by both biological assay and the determination of carotene that machine-dried alfalfa had a higher vitamin A value than field cured. The degree of difference was determined by the time and condition of exposure in the field. They found machine-dried products to contain two to 10 times the carotene content of the field cured. Hauge and Aitkenhead (1931) compared two processes of artificial drying with field curing of alfalfa. They reported that artifi-

cial drying tends to preserve the vitamin A content of alfalfa while field curing tends to destroy the vitamin A.

Studies have been made to determine the factors involved in the destruction or preservation of vitamin A in alfalfa hay. Hauge and Aitkenhead (1931) stated that high temperatures, such as are used in mechanical driers, are not destructive to vitamin A and also that the sun's rays (ultra-violet rays) are not responsible for the destruction of vitamin A during the field-curing process. They reported that enzymes are the important factor in vitamin A destruction during field curing. Conditions in field-curing processes are favorable to enzymic activity, while conditions in mechanical-drying processes are adverse to enzymic activity. Therefore the former processes tend to destroy and the latter tend to preserve vitamin A.

In 1935 Hauge reported:

> By immediate inactivation of the enzymes in alfalfa it was possible to produce dried alfalfa of very high vitamin A potency. The digestion, at 37° C., of alfalfa in which the natural enzymes have been inactivated, resulted in little or no deterioration, while digestion of such material to which active enzymes have been added resulted in a marked destruction of this factor. In samples containing the active plant enzymes, which were treated at temperatures which influence enzymic activity, there was found to be a direct correlation between the effect of temperature on the destruction of vitamin A and the effect of temperature on enzymic activity.

He also reported that enzymes were directly responsible for carotene destruction in alfalfa during the curing process and the effect of the sun's rays was indirect in that it produced temperatures which hastened enzyme activity.

Guilbert (1935) reported that inactivation of enzymes by autoclaving reduced but did not eliminate carotene loss during subsequent natural drying. He reported also that temperature was the major factor causing

variation in carotene losses in alfalfa hay and meal while in storage.

MATERIAL AND METHODS

Excellent facilities were available for work on this problem. Greenhouse space, field plots and equipment were furnished by the Department of Agronomy for studies of the various methods of handling hay. Carotene determinations were made under the supervision of the Department of Chemistry in their laboratories.

All of the field-cured samples were collected from a field of Kansas Common alfalfa which had been planted in 1937 at the Agronomy Farm on Marshall type soil. Approximately 50 g of leaves were collected in a kraft paper bag for each sample. The collection was made as quickly as possible and taken to the chemistry laboratory for weighing, moisture determination, and carotene analysis. Some samples were obtained from Kansas Common grown in the greenhouse. Unless otherwise stated, the material was collected from the field.

All of the various methods of sampling will be discussed in the section on experimental results, as a study of sampling technique was made a part of these investigations.

In 1943 the carotene determinations were made by the modified Peterson-Hughes-Freeman (1937) method which consists of an alcoholic potash saponification followed by repeated extraction with petroleum ether; then the xanthophyll is removed with 90 percent methanol and the remaining color is read as carotene on the photo-electric photometer.

In 1944 the carotene determinations were made by a modification of the Wall and Kelley(1943) method as worked out by Silker, Shrenk, and King (1944)

which consists of extracting the fresh sample in the Waring blendor, using a mixture of alcohol and Skellysolve B. After washing the extract with water to cause the alcohol layer to separate and the removal of the water alcohol layer with Skellysolve B, the combined extracts were concentrated and the pigments adsorbed on a column of magnesia and Ryflo Super-Cel. The carotene was separated from other pigments by using a five-percent solution of acetone in Skellysolve B and read on the Beckman quartz spectro photometer.

EXPERIMENTAL RESULTS

The work herein reported concerned studies involving methods of sampling alfalfa hay for carotene determinations, the effect of atmospheric conditions upon the carotene content of alfalfa hay as it cured, and the effect of various hay-making practices upon the carotene content of the cured hay.

Sampling Technique

Since very little information was available on methods of sampling alfalfa for carotene determination, it was decided to study several sampling methods.

Whole Plant. In five samples of whole plants the ratio of leaves to stems varied from 0.517 to 0.585. Table 1 shows that for an average of nine samples, the leaves contained 4.85 times more carotene than the stems and that the ratio varied among the nine samples from 3.28 to 7.36 mg per 100 g of dry matter. Since the leaves contained the larger amount of carotene and the ratio of leaves to stems varied considerably, it appeared that it would

be difficult to obtain accurate results by using whole plant samples. Figure 1 shows that the loss of carotene from the stems follows the same trend as the loss from leaves. Therefore any practice which would conserve the carotene of the leaves would probably also conserve it in the stems.

Table 1. Carotene content of leaves and stems of alfalfa.

	Milligrams carotene per 100 g of dry matter		
	Leaves	Stems	Ratio
	45.34	10.00	4.53
	43.97	9.38	4.68
	16.31	4.98	3.28
	17.42	4.80	3.63
	32.40	4.40	7.36
	29.99	6.36	4.72
	51.74	9.70	5.33
	53.32	9.84	5.42
	43.66	9.31	4.69
Average	37.13	7.64	4.85

Comparison of New and Old Leaves. The purpose of this experiment was to determine, if possible, whether the age of the alfalfa leaf had any influence on the carotene content of the leaves.

Old leaves were those which were located along the main stems of the alfalfa plant. New leaves were those which were located at the tips of the main stems and those on the small branches which grow in the leaf axil.

Samples were taken in both 1943 and 1944. The 1943 samples were from alfalfa in one-tenth to one-half bloom stage and the determinations were made on fresh material. In 1944 sample 10 was from alfalfa in pre-bud stage and sample 11 was from alfalfa in very young, lush growth about

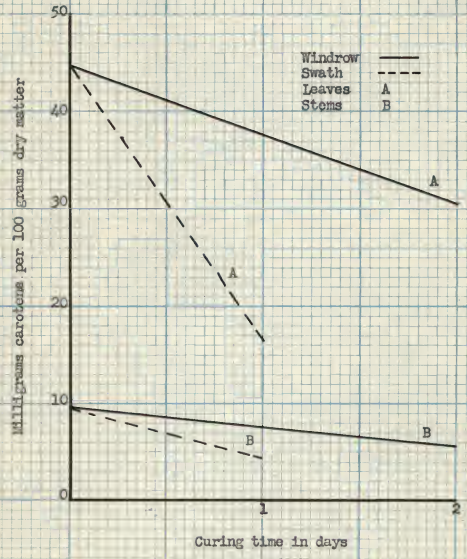

Fig. 1. The effect of curing methods upon the loss of carotene in the leaves and stems of alfalfa.

eight inches high. These samples were dried in the air oven, ground in the Wiley mill and stored at three degrees Centigrade for two months.

Table 2 shows the results of this study. The old leaves contained more carotene in all cases except one. These results indicate that carotene was built up as the leaves matured. The difference between samples 10 and 11 indicates that as the plant became more mature, the leaves contained larger amounts of carotene.

Table 2. Carotene content of new and old alfalfa leaves.

Date	Sample No.	Milligrams per 100 g dry matter		
		New	Old	Difference
1943				
Aug. 9	1	51.41	58.07	6.66
	2	50.30	57.02	6.72
	3	45.20	52.98	7.78
	4	41.62	48.80	7.18
	5	42.16	36.53	-5.63
Aug. 13	6	58.33	91.96	33.63
	7	51.67	74.80	23.13
	8	60.71	77.80	17.09
	9	51.74	57.24	5.50
1944				
Aug. 14	10[1]	17.80	25.20	7.40
	11[2]	13.30	17.80	4.50

[1]Mature plants in bud stage.
[2]Immature plants eight inches high, very lush growth.
1943 - Fresh material direct from field.
1944 - Material dried in air oven, ground in Wiley mill and stored at 3° C. for two months.

Many workers have reported that the carotene content of young alfalfa was much greater than that of alfalfa in the mature stages. Salmon, Swanson, and McCampbell (1925) reported that at bud stage, one-tenth bloom, and full bloom the percent of leaves of alfalfa was 53.4, 51.1, and 48.4 percent, respectively. This reduction in percentage of leaves as the plants grow

older is probably the reason for the reduction in carotene. The data in Table 1 show that leaves contain 4.85 times more carotene than the stems. Therefore, any reduction in amount of leaves in hay would reduce the amount of carotene, in spite of the fact that this work indicates that the amount of carotene increases as the leaves mature.

Stripping the Alfalfa. By this method the stem of the plant was grasped between the thumb and forefinger, at or near the base. Then by drawing the hand upward, the leaves and tips of the stems were removed. This proved to be a satisfactory method of sampling fresh green material in the field. With seven sets of field duplicates the standard deviation of the difference between duplicates was 2.22 mg per 100 g of dry matter. However, this method made it difficult to obtain comparable samples as the hay cured, because at the beginning of the curing period the samples contained approximately 10 percent of stems and as the hay cured the percentage of stems progressively decreased to approximately 0.0.

Leaves Only. The leaves were picked from the petiole by hand and thus comparable samples could be taken throughout the curing period; that is, no larger proportion of stem was obtained at one stage in the curing period than at another. Nine sets of field duplicates showed a standard deviation of difference between duplicates of 2.34 mg of carotene per 100 g of dry matter.

Variability Among Field Samples Compared With Laboratory Duplicates. Analyses in 1944 of 50 sets of chemistry laboratory duplicates of fresh leaves indicated that a standard deviation of difference between duplicates of 0.78 mg of carotene per 100 g of dry matter should be attributed to laboratory technique. In 1943, from analyses of 11 sets of chemistry laboratory

duplicates of fresh leaves, it was found that a standard deviation of difference between duplicates of 1.16 mg of carotene per 100 g of dry matter should be attributed to laboratory technique, or an average standard deviation of difference between duplicates of 0.97 mg per 100 g of dry matter that should be attributed to laboratory technique.

The standard deviation of the difference between duplicates of different methods of field sampling was found to be 2.34 mg per 100 g of dry matter for leaves only and 2.22 mg for the stripping method. The variability in these two methods of field sampling included both the variability due to field sampling and that due to laboratory sampling. The standard deviation of the difference between duplicates in laboratory sampling was slightly less than one mg per 100 g of dry matter, and that for the field sampling was approximately two and one-fourth mg per 100 g dry matter. Of the total field sampling variability, approximately 40 percent was due to laboratory technique. Thus the variability due to collecting samples in the field was slightly greater than the variability in laboratory technique.

The causes for the smaller deviation in 1944 as compared to 1943 were because of improved laboratory technique in weighing, drying, and of methods of carotene determinations.

Effect of Atmospheric Conditions

Humidity. An experiment was conducted to determine the effect of different relative humidity percentages upon the preservation or destruction of carotene. A series of small desiccators was set up with different percentages of sulphuric acid to give the humidity percentage desired. The percent of sulphuric acid necessary was calculated from the method reported

on by Wilson (1921) who gave a graph and stated the thermodynamic theory for calculation. The desiccators were arranged in quadruplicate at 0.0, 20 and 80 percent humidity. A fourth set of desiccators was set up without acid and the humidity was estimated from 80 to 100 percent. The desiccators were placed in the greenhouse, one-half of them being protected from the light by tight cardboard cartons.

The sampling was done by picking a large amount of leaves from plants of Kansas Common alfalfa which was growing in the greenhouse. These leaves were thoroughly mixed and 10 g samples weighed out for the experiment. As soon as the samples were weighed they were placed in the desiccators, taken to the greenhouse, and allowed to cure for 43 hours. The samples in the light received approximately 10 hours of bright sunlight during the curing period. The exact temperatures inside the desiccators were not known; however, it was known that the samples in the sun were subjected to higher temperatures than were the samples in the dark.

Table 3 shows the milligrams of carotene remaining under the various conditions. The analysis of variance was run on these data by Paterson's (1939) method and Table 4 shows the computation of this relationship.

A difference between means (Table 5) greater than 1.20 was significant at the five-percent level, and a difference greater than 1.74 was significant at the one-percent level. The data show that there was no significant difference between any of the humidities in the dark. Also, the data show that there was no significant difference between 80-100 percent, 80 percent, and 20 percent humidity in the light, but there was a significant difference between 0.0-percent humidity and any one of the above-mentioned humidities.

Table 3. Effect of relative humidity on the amount of carotene retained during the curing of alfalfa leaves in the light and dark.

Humidity percent	Milligrams carotene per 100 g		
	Light	Dark	Average
0.0	5.98	9.39	
0.0	6.66	8.38	
Average	6.32	8.89	7.60
20	4.45	7.53	
20	3.78	8.61	
Average	4.12	8.07	6.09
80	3.83	7.72	
80	4.24	8.06	
Average	4.04	7.89	5.96
80-100	4.29	8.38	
80-100	4.86	7.55	
Average	4.58	7.97	6.27
Average, all humidities	4.76	8.20	

Table 4. Analysis of variance table for humidity studies.

Factor	d.f.	Sum of squares	Variance	Calculated F	Table reading of F (P=0.01)
Total	15	57.6603			
Between humidities	3	6.8883	2.2961	8.3604	7.59
Between light and dark	1	47.3609	47.3689	172.4386	11.26
Interaction humidity x light and dark	3	1.2055	0.4018	1.4627	7.59
Within humidities	8	2.1974	0.2747		

Table 5. Average milligrams of carotene per 100 g retained at the various humidities in the light and dark.

Treatment	Percent relative humidity			
	80-100*	80	20	0.0
Light	4.58	4.04	4.12	6.32
Dark	7.97	7.89	8.07	8.89

*Desiccators without acid or water.

During the time that the field-curing experiments were being carried on, the relative humidity range in the field was from 20 to 100 percent. For some of the most drying days during the summer of 1944 the relative humidity ranged from a minimum of 30 to a maximum of 60 percent, with an average for the day around 54 percent. For some of the most humid days the relative humidity range was from a minimum of 80 to a maximum of 100 percent, with an average of 96 percent for the day.

It would appear from these experiments that relative humidity as found in the field affects the retention or destruction of carotene only in that it shortens or lengthens the curing period.

Temperature. The effect of temperature on preservation or destruction of carotene was determined in desiccators in which humidity was controlled through the use of sulphuric acid as explained in the section on effect of humidity.

Experiments were conducted in both 1943 and 1944 to determine the effect of temperature on the loss of carotene content of alfalfa leaves during a 24-hour curing period in the light and dark. In the dark the humidity was held at 20 percent. The data in Table 6 show that temperature had a very marked effect on the loss of carotene. The higher the temperature the greater the losses. In the light no attempt was made to keep a constant tempera-

ture. The temperature range for the 24-hour curing period on June 27 was 50° to 94° F. for the desiccator which was cooled in an ice and brine solution, and 80° to 156° F. for the desiccator which was left without any cooling. On August 10 the temperature range for the 24-hour curing period was 50° to 94° F. for the desiccator which was cooled and 80° to 166° F. for the uncooled desiccator.

Table 6. The effect of temperature on the loss of carotene in alfalfa leaves cured in the dark.

Temperature	Milligrams carotene per 100 g		
	5/20/43	6/21/44	Average
125° F.	3.15	1.70	2.43
70°-72° F.	5.92	6.85	6.39
43° F.	10.92	12.25	11.59
Original material	12.64	13.03	12.84

The data in Table 7 indicate that temperature had a marked effect upon the loss of carotene in the light. The difference between the carotene readings, namely, 1.6 and 0.8, at the two high temperatures, probably is accounted for by the fact that on June 27 the maximum temperature was 156° F. and on August 10 it was 166° F. On June 27 the temperature out-of-doors was 97° F. and on August 10 the temperature was 100° F. Both days were clear and very bright.

The original green material in all the temperature studies was almost identical. These data indicate that high temperatures are favorable to carotene destruction whether leaves are cured in the light or in the dark.

Table 7. The effect of temperature on the loss of carotene in alfalfa leaves cured in the light.

Temperature	6/27/44 Mg carotene per 100 g	Temperature	8/15/44 Mg carotene per 100 g	Average
80°-156° F.	1.6	80°-166° F.	0.8	1.2
50°-94° F.	7.6	50°-94° F.	7.7	7.65
Original material	13.03		13.6	13.32

Light. The data in Table 4 show a very highly significant difference in the loss of carotene in alfalfa leaves cured in the light and dark; however, it was known that the samples cured in the light were subjected to considerable higher temperatures than those cured in the dark. The data in Tables 6 and 7 show clearly that temperature had a very marked effect on carotene losses whether in the light or dark. The differences in amount of carotene retained in the light and dark in Table 3 can be largely accounted for by the differences in temperature.

More work is necessary before definite conclusions can be drawn on this question.

Effect of Curing Methods in the Field

This series of experiments was set up to study the preservation or destruction of the carotene content of alfalfa in various methods of field curing.

For simplification the practices will be referred to as follows: "Swath" refers to hay as it was cut down by the mower. "Windrow A" refers to hay in the windrow which was raked immediately after mowing. "Windrow B"

was raked after the hay had wilted in the swath for from two to four hours. "Cock A" refers to hay which was put into the cock immediately after mowing, and "cock B" to that which had wilted in the swath for from two to four hours before being placed in the cock.

In view of the fact that the leaves contain a large percentage of the carotene of the plant, all of the carotene and dry matter determinations were made on the leaves. In all cases the samples were from the alfalfa variety Kansas Common grown on the Agronomy Farm.

In all experiments the samples were taken as follows: The swath samples were taken from an area one mower-swath wide and approximately two feet long. The windrows were one to one and one-half feet in diameter, raked with a side-delivery rake. The windrow samples were taken from a cross section of the windrow six to 12 inches long. The cock samples were three or four handfuls of hay from as many locations inside the cock. These contained no material which was exposed to the sun after the cock was built. The cocks were two and one-half to three feet in diameter at the base and two to three feet high when built.

Tables 8, 9 and 10 show the carotene determinations, dry matter data, and weather conditions, respectively.

One experiment was started on August 17, 1943, on alfalfa in one-tenth bloom stage in which swath, windrow "A" and cock "A" were compared. The alfalfa was cut at 9:00 A.M. and raked into the windrow by 9:30, and the cock was built immediately after the hay was raked. The swathed hay cured a day sooner than the windrowed hay but contained about 14 mg less carotene per 100 g of dry matter. The hay in the cock cured slower than hay in the swath or windrow and, due to unfavorable weather conditions with a light shower,

Table 8. The effect of method of handling hay in the field on the loss of carotene from alfalfa leaves during curing.

Date	Time sampled	Hours after cutting	Milligrams carotene per 100 g of dry matter				
			Swath	Windrow		Cock	
				Immediately	After 2-4 hrs. wilting	Immediately	After 2-4 hrs. wilting
1943							
Aug. 17	9:00 A.M.	0	44.66	44.66		44.66	
17	10:30 A.M.	1	42.57				
17	2:30 P.M.	5	41.84	40.43		40.94	
17	7:30 P.M.	10	30.90	32.00		39.71	
18	7:30 A.M.	22	34.90	36.69		35.81	
18	2:30 P.M.	29	16.87*				
19	2:30 P.M.	53		31.20		29.25	
23	2:30 P.M.	149				18.65**	
1944							
May 22	10:30-11:00 A.M.	0	50.4	50.4		50.4	
22	1:00-2:30 P.M.	3	44.0	53.4	44.0	48.9	44.0
23	9:00-10:00 A.M.	22-23	24.3	41.2	39.8	37.8	37.0
23	2:00-4:00 P.M.	28-30	16.0	35.2	46.2		
24	1:00-4:00 P.M.	50-52	6.2	28.7	10.1	30.6	19.5
25	1:00-4:00 P.M.	75-79	4.8	13.1	15.9	11.7**	11.8
June 5	9:00-9:30 A.M.	0	49.8	49.8		49.8	
5	1:00-4:00 P.M.	4-8	44.4	48.4	44.4	46.4	44.4
6	8:00-9:00 A.M.	23	30.5	32.5	33.2		
6	1:00-4:00 P.M.	28-32	25.8	30.9	34.3	40.9	34.5
13	1:00-4:00 P.M.	196-200	0.8	4.5	6.1	13.6**	7.4**
June 19	11:00 A.M.	0	45.4	45.4		45.4	
19	3:00-4:00 P.M.	4	31.0	43.7	31.0		31.0
20	1:00-4:00 P.M.	26-30	9.8	28.6	23.5	23.1	20.9
21	1:00-4:00 P.M.	50-54	8.4	13.5	11.1	17.8	13.7
22	1:00-4:00 P.M.	74-78	.6	13.5	11.3	14.5	11.1
July 13	10:00 A.M.	0	52.6	52.6		52.6	
13	2:30 P.M.	4	48.8		48.8		48.8
14	1:00-4:00 P.M.	27-31	12.0	26.1	29.6	25.7	17.5

*The figures underscored indicate when the sample was ready to put up.
**Cocks were tough, moldy, spoiled.

Table 9. The effect of methods of handling hay in the field on the percent dry matter, in the leaves, during the curing process.

Date	Time sampled	Hours after cutting	Swath	Percent dry matter in the leaves			
				Windrow		Cock	
				Immediately	After 2-4 hrs. wilting	Immediately	After 2-4 hrs. wilting
1943							
Aug. 17	9:00 A.M.	0	36.5	36.5		36.5	
17	10:30 A.M.	1	44.5				
17	2:30 P.M.	5	57.0	53.5		48.0	
17	7:30 P.M.	10	82.7	69.4		50.0	
18	7:30 A.M.	22	53.4	62.7		50.0	
18	2:30 P.M.	29	93.0*				
19	2:30 P.M.	53		82.7		50.0	
23	2:30 P.M.	149				77.0	
1944							
May 22	10:30-11:00 A.M.	0	27.2	27.2		27.2	
22	1:00-2:30 P.M.	3	48.0	32.0	48.0	35.4	48.0
23	9:00-10:00 A.M.	22-23	75.3	37.4	69.3	40.0	50.0
23	2:00-4:00 P.M.	28-30	92.0	45.4	53.3		
24	1:00-4:00 P.M.	50-52	91.4	44.2	91.4	34.0	67.3
25	1:00-4:00 P.M.	74-78	92.0	89.3	89.3	81.3	86.0
June 5	9:00-9:30 A.M.	0	28.0	28.0		28.0	
5	1:00-4:00 P.M.	4-8	49.4	38.6	49.4	34.7	49.4
6	8:00-9:00 A.M.	23	74.0	64.0	62.0		
6	1:00-4:00 P.M.	28-32	85.9	69.3	76.7	40.6	69.0
13	1:00-4:00 P.M.	196-200	90.3	89.5	88.0	66.0	87.0
June 19	11:00 A.M.	0	26.7	26.7		26.7	
19	3:00-4:00 P.M.	4	70.0	38.0	70.0		70.0
20	1:00-4:00 P.M.	26-30	60.0	36.6	60.0	43.3	64.6
21	1:00-4:00 P.M.	50-54	89.4	59.3	81.4	42.2	73.2
22	1:00-4:00 P.M.	74-78	93.0	89.9	91.2	92.0	90.2
July 13	10:00 A.M.	0	29.3	29.3		29.3	
13	2:30 P.M.	4	58.0		58.0		58.0
14	1:00-4:00 P.M.	27-31	92.7	71.0	92.0	52.5	88.0

*The figures underscored indicate when the sample was ready for storage.

was not cured sufficiently for storage until five days after cutting. By this time it had molded and was unfit for feed; however, it still contained slightly more carotene than the hay cured in the swath when it was stored.

Table 10. Weather conditions during the process of curing alfalfa in the field.

Date	Temperature, degrees F.		Wind		Sun	Rain
	Maximum	Minimum	Miles	Direction		(inches)
1943						
Aug. 17	80	50	63	W	Clear	0.20
18	78	44	41	N	"	—
19	83	52	21	NE	"	—
20	90	61	83	SE	"	.11
21	87	62	58	E	"	—
22	100	67	99	S	"	—
23	99	80	153	S	"	—
1944						
May 22	85	57	56	SW	Clear	.06
23	85	61	46	SW	Partly cloudy	—
24	86	57	43	SE	Clear	—
25	90	68	83	SW	Partly cloudy	—
June 5	86	52	142	NW	Clear	.98
6	76	47	138	NW	"	—
7	70	50	55	SE	Cloudy	—
8	70	58	126	SE	"	.94
9	74	57	84	NW	"	.07
10	67	53	40	SE	"	—
11	82	62	110	S	Clear	—
12	87	67	106	SE	Cloudy	—
13	83	60	74	SW	Clear	—
June 19	85	62	54	N	Clear	.34
20	77	57	70	SE	Cloudy	.50
21	81	58	127	SW	Clear	—
22	97	73	114	SW	"	—
July 13	82	58	6	NE	Clear	—
14	86	59	78	NE	"	—
15	92	69	124	E	Partly cloudy	1.95
16	83	67	30	E	Cloudy	1.58
17	77	65	21	SE	Clear	.46

Due to the trouble encountered in curing the hay which was windrowed and cocked immediately after mowing, two other methods were added in 1944. They were windrowing and cocking the hay after it had wilted in the swath for from two to four hours. The length of time the hay was allowed to wilt in the swath depended upon weather conditions.

On May 22, 1944, a plot of alfalfa in one-tenth bloom stage was cut at 10:30 A.M. and the windrow "A" raked and cock "A" built by 11 o'clock. Three hours after the hay was cut, windrow "B" was raked and cock "B" was built. The hay from windrow "B" was cured sufficiently for barn storage at the same time as the hay in the swath and had retained approximately three times as much carotene. The hay in windrow "A" and cock "A", and in cock "B", required an additional day for curing before they were ready for storage and were not as high in carotene as windrow "B". The hay in cock "A" was slightly molded.

A plot of alfalfa was again cut on June 5 in one-tenth to one-half bloom stage. The mowing was completed by 9:00 A.M. Windrow "A" was raked by 9:15 and cock "A" built by 9:30. Four hours later windrow "B" was raked and cock "B" built. By the afternoon of June 6 the swath and windrow "B" were cured sufficiently for barn storage. As shown in Table 8, windrow "B" was higher in carotene content than the swath. The weather conditions on June 7, 8 and 9 (Table 10) were unfavorable for hay curing. By June 13 cock "B" and windrow "A" were dry enough for storage. Both had lost a great deal of carotene and were much lower than the swath was when it was cured enough for storage. Cock "A" was molded beyond any use as feed even though it had retained more carotene than windrow "A" and cock "B".

On June 19, alfalfa in the prebud stage was mowed by 11 o'clock.

Windrow "A" was raked and cock "A" built by 11:20. After three and one-half hours of wilting in the swath, windrow "B" was raked and cock "B" was built. The following day, June 20, was cloudy and one-half inch of rain fell. The 21st was a clear, windy, drying day, and by afternoon the swath and windrow "B" were ready for storage. The windrow had lost a large percentage of its carotene but it still contained considerable more than the swath. An additional day was required to cure cocks "A" and "B" and windrow "A". In this case both windrow "A" and cock "A" retained more carotene than windrow "B". However, an additional day was required for curing, which always increases the weather hazard.

Alfalfa in one-tenth bloom stage was cut by 10 o'clock July 13. Again windrow "A" and cock "A" were constructed as soon after mowing as possible. They were completed in half an hour. Windrow "B" and cock "B" were constructed after three and one-half hours wilting in the swath. By the afternoon of the 14th the swath and windrow "B" and cock "B" were cured and ready for storage. The windrow had retained two and one-half times as much carotene as the swath and one and seven-tenths times as much as cock "B". The rainfall on July 15, 16, and 17 was 1.93, 1.58, and 0.46 inches, respectively, so the experiment was terminated because windrow "A" and cock "A" molded and were of no value.

Investigation in methods of field curing of alfalfa hay indicated that cocking hay prolongs the curing period, thus increases the weather hazard which is clearly pointed out on May 22, June 5, and July 13 in Table 8. The same problem is encountered in windrowing immediately after the mower. The results also show that swathed hay, because it is left exposed, cures and loses its carotene rapidly. Hay windrowed after three or four hours of wilt-

ing in the swath was cured and ready to be stored just as soon as the swathed hay, and retained much more carotene. In three cases out of four, hay windrowed after three to four hours wilting in the swath retained more carotene and in nearly all instances it was cured at least a day or more before either of the cocks or the windrow raked immediately after mowing.

DISCUSSION

In the determination of carotene in alfalfa it appears at present that fresh green alfalfa in the field may be sampled equally well by either stripping the plants or collecting only leaves. The variability in the leaf and stem ratio, in addition to the fact that leaves contain three to seven times as much carotene as the stems, makes whole plant sampling undesirable.

The sampling of hay as the curing process progressed was done by collecting leaves only. The stripping method involved the problem of getting approximately 10 percent of stems in the sample at the beginning and 0.0 percent of stems at the end of the curing period, which would make the results less reliable.

The results on new and old leaves indicate that carotene is continually being built up in the leaf, at least until the blooming period; but more study is needed before conclusions can be drawn.

Hauge and Aitkenhead (1931) and later work by Hauge (1935) showed that enzymes were directly responsible for the destruction of carotene during the field-curing process. On the basis of this hypothesis, humidity, temperature and light have only an indirect effect by producing conditions favorable or unfavorable for enzymic activity.

Relative humidity as found in the field during the summer in Kansas

would appear to be effective in carotene preservation or destruction only insofar as it shortens or lengthens the time required for completion of the curing process.

In the field curing of hay, high temperatures tend to increase carotene destruction while lower temperatures tend to decrease the destruction of carotene.

This work indicates that light may have some effect on carotene destruction. However, carotene tended to be destroyed at high temperatures and tended to be preserved at lower temperatures either in the light or the dark. More information is necessary before definite conclusions can be reached.

The results obtained in the experiments on field curing of hay indicated that prolonged curing of hay in the swath greatly increased the destruction of carotene. It is generally known that prolonged swath curing will often produce over-curing which increases the loss of leaves, further decreasing the quality of alfalfa hay.

Windrowing alfalfa hay after it has wilted in the swath for from two to four hours helps to preserve the carotene and requires no longer time than curing in the swath. Windrowing immediately after mowing also preserves the carotene, but it prolongs the curing period and thus increases the weather hazard. However, during drought years this practice may have some advantages.

Cocking hay either immediately after mowing or after the hay has wilted in the swath for from two to four hours helps preserve the carotene. However, results indicate that, as a curing practice, it prolongs the curing period. It was found in many of the experiments conducted on cocked hay that, by prolonging the curing time, rainy weather was encountered which in

most cases caused spoilage of the hay beyond any forage value.

SUMMARY

In this study experiments were conducted to determine the most satisfactory method of sampling alfalfa hay in the field for carotene determinations, and to determine the practice of haying that would reduce losses of carotene to a minimum.

The use of leaves only was found to be the most satisfactory method of sampling alfalfa hay for carotene determination throughout the field-curing period.

Temperature was found to be the chief atmospheric factor in causing variation in loss of carotene in alfalfa hay during the curing process. Relative humidity had no effect other than lengthening or shortening the curing period.

Results indicate that the older leaves from a plant contained more carotene than new leaves.

Allowing hay to wilt in the swath for from two to four hours and then raking it into a small windrow was found to be the best practice from the standpoint of rapid curing and preservation of carotene.

Swath-cured hay required as long time for curing as did the windrow which was raked after wilting in the swath for from two to four hours but always contained much less carotene.

Windrowing immediately after mowing preserved the carotene but prolonged the curing time which increased weather hazards.

Cocking hay either immediately after mowing or after wilting in the swath for from two to four hours cannot be recommended as hay curing prac-

tices in Kansas because these practices prolong the curing period and increase weather hazards.

ACKNOWLEDGMENT

This study was conducted through the cooperation of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture; the College Departments of Agronomy and Chemistry; and the Kansas Industrial Development Commission. Special acknowledgment is given to Mr. C. O. Grandfield for helpful suggestions and use of equipment; to Dr. H. H. Laude, who acted in the capacity of major instructor; to Dr. J. S. Hughes, Dr. Ralph H. Silker, and Instructor M. J. Caldwell for helpful suggestions and use of equipment concerning chemical work, and to Mr. W. H. Rhodes for assistance in conducting the experiments.

LITERATURE CITED

Dechdel, S. I., Clyde, A. W., Cromer, C. O., and Williams, P. S.
Dehydrated versus sun-cured alfalfa for milk production. Penn. Agr. Expt. Sta. Bul. 396. 24 p. 1940.

Dexter, S. T. and Moore, L. A.
Carotene in alfalfa hay. Mich. Agr. Expt. Sta. Qr. Bul. 20: 75-76. 1937.

Douglass, Earl, Tobiska, J. W., and Vail, G. E.
Studies on changes in vitamin content of alfalfa hay. Colo. Agr. Expt. Sta. Tech. Bul. 4. 63 p. 1933.

Guilbert, H. R.
Factors affecting the carotene content of alfalfa hay and meal. Jour. Nut. 10: 45-62. 1935.

Hauge, Sigfred M.
Vitamin A value of alfalfa cut at different stages of maturity. Assoc. Offic. Agr. Chem., Jour. 17: 304-307. 1934.

Mauge, Sigfred M.
Evidence of enzymic destruction of the vitamin A value of alfalfa during the curing process. Jour. Biol. Chem. 108: 331-336. 1935.

Mauge, Sigfred M. and Aitkenhead, Wm.
Effect of artificial drying upon the vitamin A content of alfalfa. Jour. Biol. Chem. 93: 657-665. 1931.

Kane, Edward A., Wiseman, Herbert G., and Cary, C. A.
Loss of carotene in hay and alfalfa meal during storage. Jour. Agr. Res. 55: 837-847. 1937.

Kiesselbach, T. A. and Anderson, Arthur.
Quality of alfalfa hay. U. S. Dept. Agr. Tech. Bul. 235. 25 p. 1931.

Morrison, F. B.
Feeds and feeding. 20th ed. 1050 p. New York. Morrison Publishing Co. 1936.

Peterson, D. D.
Statistical technique in agricultural research. New York. McGraw-Hill. 263 p. 1939.

Peterson, W. J., Hughes, J. S., and Freeman, H. P.
Determination of carotene in forage - modification of the Guilbert Method. Ind. Engrg. Chem., anal. ed. 9: 71-72. 1937.

Russell, Walter C., Taylor, M. W., and Chichester, D. F.
Effect of the curing process upon carotene and vitamin A content of alfalfa. N. J. Agr. xpt. Sta. Bul. 560. 8 p. 1934.

Salmon, S. C., Swanson, C. O., and McCampbell, C. W.
Experiments relative to time of cutting alfalfa. Kans. Agr. Expt. Sta. Tech. Bul. 15. 50 p. 1925.

Silker, Ralph E., Shrenk, W. G., and King, H. H.
Carotene content of alfalfa retention on dehydration and storage. Ind. and Engrg. Chem. 36: 831-834. 1944.

Smith, Margaret Cammack.
The effect of storage upon the vitamin A content of alfalfa hay. Jour. Agr. Res. 53: 681-684. 1936.

Snyder, W. W. and Moore, L. A.
The carotene contents of several herbages during the growing season. Jour. Dairy Sci. 23: 369-371. 1940.

Vail, G. E., Tobiska, J. W., and Douglass, Earl.
Vitamins in alfalfa hay. Colo. Agr. Expt. Sta. Tech. Bul. 18. 19 p. 1936.

Wall, M. E. and Kelley, E. C.
Determination of pure carotene in plant tissue. Ind. Engrg. Chem., anal. ed. 15: 18-20. 1943.

Wallis, C. C.
Factors affecting the vitamin A and D potency of alfalfa hay. Jour. Dairy Sci. 25: 685-687. 1942.

Wilson, P. E.
Humidity control by means of sulphuric acid solution. Ind. and Engrg. Chem. 13: 326. 1921.

Woods, Ella, Atkeson, F. W., Willhouson, Harry, and Johnson, R. F.
Vitamin A activity of third-cutting alfalfa hay as affected by methods of curing. Jour. Dairy Sci. 19: 591-596. 1936.